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Thesis

THE INTERNAL SECRETION OF THE OVARIES

by

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INTRODUCTION.

"WOMAN is because of her ovary. All her somatic and psychic characteristics, her primary and secondary sexual qualities are due to her ovarian activities." *

This paper deals with the internal secretions of the ovary and the corpus luteum. The ovary has two kinds of secretions, one external and the other internal. The external secretion is in the form of an ovum which is discharged periodically every twenty-eight days by the rupture of a Graafian follicle. If the ovum is fertilized, it receives nidation in the uterus, if not, it is discharged with the menstrual flux by the vagina.

That the ovary also secretes an internal secretion seems certain because of the fact that all the feminine characteristics are developed long before the maturity of the ova or the development of the corpus luteum. The feminine characteristics are suppressed and changed by ovariotomy which points to the conclusion that an internal secretion is poured forth into the blood which determines and maintains the female sex characteristics. That the corpus luteum also secretes an internal secretion is proved by the fact that, when the corpora lutea are destroyed, the fertilized ovum does not appear to be able to attach itself to the wall of the uterus, and the inhibitory action upon ovulation is stopped.

In the preparation of this paper I have endeavored to keep in mind two guiding principles; first, the importance of simplicity and clarity in the presentation of the facts and theories, and second,

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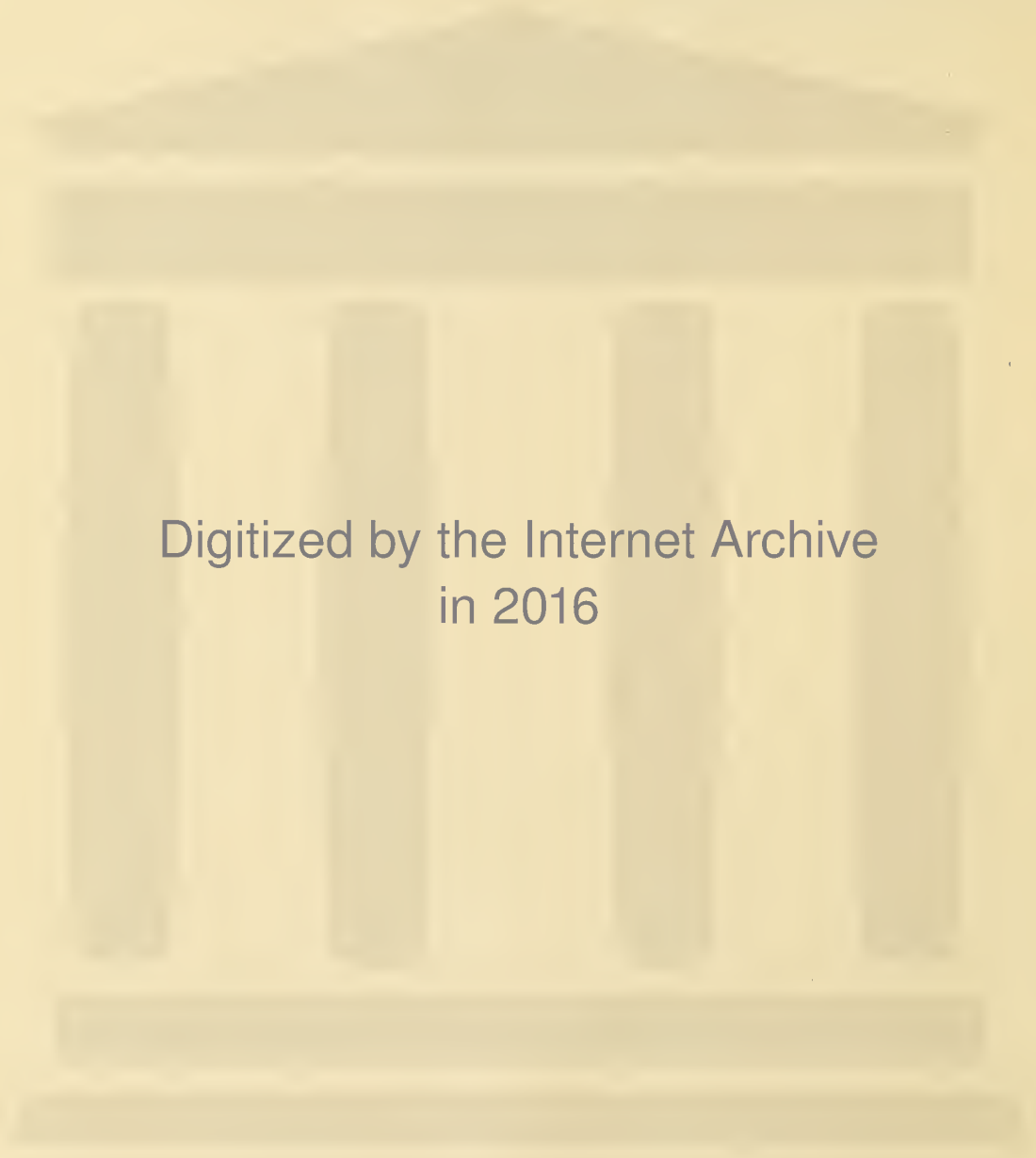
* Anonymous.

the necessity of a reasonable limitation of the subject-matter selected. There was also the need of thoroughly examining the literature and to emphasize those conclusions which seemed to be the most justified by experimentation and observation. I found it necessary to make the desired reduction in material by a process of elimination rather than by condensation.



PART II. Gross Anatomy.

The ovaries are homologous with the testes in the male. They are two nodular bodies, situated one on either side of the uterus in relation to the lateral wall of the pelvis, and attached to the back of the broad ligament of the uterus, behind and below the uterine tubes. The ovaries are of a grayish-pink color, and present either a smooth or a puckered uneven surface. They are each about 4 cm. in length, 2 cm. in width, about 8 mm. in thickness, and weigh from 2 to 3.5 grams. Each ovary presents a lateral and a medial surface, an upper or tubal and a lower or uterine extremity, and an anterior or mesovarian and a posterior border. It lies in a shallow depression, named the ovarian fossa, on the lateral wall of the pelvis. This fossa is bounded above by the external iliac vessels, in front by the obliterated umbilical artery, and behind by the ureter. The ovary becomes displaced during the first pregnancy and never again returns to its original position.

In the erect posture the long axis of the ovary is vertical. The tubal extremity is near the external iliac vein, and to it are attached the ovarian fimbria of the uterine tube and a fold of peritoneum, the suspensory ligament of the ovary, which is directed upward over the iliac vessels and contains the ovarian vessels. The uterine end is directed downward toward the pelvic floor and is usually narrower than the tubal extremity. It is attached to the lateral angle of the uterus, immediately behind the uterine tube, by a rounded cord, called the ligament of the ovary, which lies within the broad ligament and contains some non-striped muscular fibers. The lateral surface is in contact with the parietal peritoneum which lines the ovarian fossa, while the medial surface is to a large extent covered by the fimbriated extremity

of the uterine tube. The mesovarian border is straight and is directed toward the obliterated umbilical artery, and is attached to the back of the broad ligament by a short fold named the mesovarium. Between the two layers of this fold the bloodvessels and nerves pass to reach the hilum of the ovary. The free border is convex and is directed toward the ureter. The uterine tube arches over the ovary, running upward in relation to its mesovarian border, then curving over its tubal pole, and finally passing downward on its free border and medial surface.

The epoöphoron lies in the mesosalpinx between the ovary and the uterine tube, and consists of a few short tubules which converge toward the ovary while their opposite ends open into the rudimentary duct, the duct of Gärtner.

The paroöphoron consists of a few scattered rudimentary tubules, situated in the broad ligament between the epoöphoron and the uterus. The short tubules of the epoöphoron and the tubules of the paroöphoron are remnants of the tubules of the Wolffian body or mesonephros; while the duct of Gärtner is a persistent portion of the Wolffian duct.

In the fetus the ovaries are situated, like the testes, in the lumbar region, near the kidneys, but they gradually descend into the pelvis.

PART III. Embryology.

The generative organs are developed from the intermediate cell-mass, which is situated between the primitive segments and the lateral plates of mesoderm. The permanent organs of the adult are preceded by a set of structures which are purely embryonic, and which, with the exception of the duct, disappear almost entirely before the end of fetal life. These embryonic structures are on either side; the pronephros, the mesonephros, the metanephros,[1] and the Wolffian and Müllerian ducts. The pronephros disappears very early and the structural elements of the mesonephros mostly degenerate, but in their place is developed the genital gland in association with which the Wolffian duct remains as the duct of the male genital gland, and the Müllerian as that of the female.

*In the outer part of the intermediate cell-mass, immediately under the ectoderm, in the region from the fifth cervical to the third thoracic segments, a series of short evaginations from each segment grows dorsalward and extents caudalward, fusing successively from before backward to form the pronephric duct. This continues to grow caudalward until it opens into the ventral part of the cloaca where beyond the pronephros it is termed the Wolffian duct. The original evaginations form a series of transverse tubules, each of which communicates by means of a funnel-shaped ciliated opening with the celomic cavity, and in the course of each duct a glomerulus is developed. A secondary glomerulus is formed ventral to each of these, and the complete group constitutes the pronephros which undergoes rapid atrophy and disappears.

On the medial side of the Wolffian duct, from the sixth cervical to the third lumbar segments, a series of tubules, the Wolffian tubules, is developed. These tubules first appear as solid masses of

1. Later becomes permanent Kidney.

* delete * to * on page 7.

cells, which later become hollowed in the center. One end of the latter grows toward and finally opens into the Wolffian duct, the other dilates and is invaginated by a tuft of capillary bloodvessels to form a glomerulus. The tubules collectively constitute the mesonephros or Wolffian body. By the fifth or sixth week this body forms an elongated spindle-shaped structure, called the urogenital fold, which projects into the celomic cavity at the side of the dorsal mesentery, reaching from the septum transversum in front to the fifth lumbar segment behind. In the latter fold the reproductive glands are developed. The Wolffian bodies atrophy and for the most part disappear coincidently with the development of the permanent kidneys. The atrophy begins during the sixth or seventh week and rapidly proceeds, so that, by the beginning of the fifth month, only the ducts and a few of the tubules remain.

In the male the Wolffian duct persists and forms the tube of the epididymis, the ductus deferens and the ejaculatory duct, while the seminal vesicle arises during the third month as a lateral diverticulum from its hinder end. A large part of the head end of the mesonephros atrophies and disappears, while, of the remainder, the anterior tubules form the efferent ducts of the testis. The posterior tubules are represented by the ductuli aberrantes, and by the paradidymis, which is sometimes found in front of the spermatic cord above the head of the epididymis.

In the female the Wolffian bodies and ducts atrophy. The remains of the Wolffian tubules are represented by the epoöphoron or organ of Rosenmüller, and the paroöphoron, two small collections of rudimentary blind tubules which are situated in the mesosalpinx. The lower part of the Wolffian duct disappears, while the upper part persists as the longitudinal duct of the epoophoron or the duct of Gärtner.

Shortly after the formation of the Wolffian ducts, a second pair

of ducts is developed which are called the Müllerian ducts. Each arises on the lateral aspect of the corresponding Wolffian duct as a tubular invagination of the cells lining the celom. The orifice of the invagination remains patent, and undergoes enlargement and modification to form the abdominal ostium of the uterine tube. The ducts pass backward lateral to the Wolffian ducts, but toward the posterior end of the embryo they cross to the medial side of these ducts, and thus come to lie side by side between and behind the latter, the four ducts forming what is termed the genital cord. The Müllerian ducts end in an epithelial elevation, the Müllerian eminence, on the ventral part of the cloaca between the orifices of the Wolffian ducts, while at a later date they open into the cloaca in this situation.*

In the male the Müllerian ducts atrophy, but traces of their anterior ends are represented by the appendices testis, while their terminal fused portions form the utriculus in the floor of the prostatic portion of the urethra.

In the female the Müllerian ducts persist and undergo further development. The portions which lie in the genital cord fuse to form the uterus and vagina. The parts in front of this cord remain separate and each forms the corresponding uterine tube, the abdominal ostium of which is developed from the anterior extremity of the original tubular invagination from the celom. The fusion of the Müllerian ducts begins in the third month, and the septum formed by their fused medial walls disappears from below upward, and thus the cavities of the vagina and uterus are produced. About the fifth month an annular constriction marks the position of the neck of the uterus, and after the sixth month the walls of the uterus begin to thicken. For a time the vagina is represented by a solid rod of epithelial cells. A ring-like outgrowth of this epithelium occurs at the lower end of the uterus and marks the future vaginal fornices. About the fifth or sixth

month the lumen of the vagina is produced by the breaking down of the central cells of the epithelium, while the hymen represents the remains of the Müllerian eminence.

The first appearance of the genital gland is essentially the same in the two sexes, and consists in a thickening of the epithelial layer which lines the peritoneal cavity on the medial side of the urogenital fold. The thick plate of epithelium extends deeply, pushing before it the mesoderm and forming a distinct projection. This is termed the genital ridge, and from it the testis in the male and the ovary in the female are developed. At first the mesonephros and genital ridge are suspended by a common mesentery, but as the embryo grows the genital ridge gradually becomes pinched off from the mesonephros, with which it is at first continuous, though it still remains connected to the remnant of this body by a fold of peritoneum, the mesorchium or mesovarium. About the seventh week the distinction of sex in the genital ridge begins to be perceptible.

The ovary, thus formed from the genital ridge, is at first a mass of cells derived from the celomic epithelium. Later the mass is differentiated into a central part or medulla covered by a surface layer, the germinal epithelium. Between the cells of the germinal epithelium a number of larger cells, the primitive ova, are found, and these are carried into the subjacent stroma by the bud-like ingrowths of the germinal epithelium called genital cords. The surface epithelium ultimately forms the permanent epithelial covering of this organ. It soon loses its connection with the central mass,* and a tunica albuginea develops between them. The ova are chiefly derived from the cells of the central mass. These are separated from one another by the growth of connective tissue cells, and in this way the rudiments of the ovarian follicles are formed.

* epithelial cells

PART IV. Histology.

The surface of the ovary is covered by a layer of columnar cells which constitutes the germinal epithelium of Waldeyer. This epithelium gives to the ovary a dull gray color as compared with the shining smoothness of the peritoneum and the transition between the squamous epithelium of the peritoneum and the columnar cells which cover the ovary is usually marked by a line around the anterior border of the ovary. The ovary consists of a number of vesicular ovarian follicles imbedded in the meshes of a stroma or framework.

The stroma is a peculiar soft tissue, abundantly supplied with bloodvessels, consisting for the most part of spindle-shaped cells with a small amount ordinarily of connective tissue. These cells have been regarded by some anatomists as unstriped muscle cells which they most resemble, by others as connective-tissue cells. Near the surface of the organ this tissue is much condensed, and forms a layer composed of short connective-tissue fibers, with fusiform cells between them. The stroma of the ovary may contain interstitial cells resembling those of the testis.

Upon making a section of an ovary, numerous round transparent vesicles of various sizes are to be seen which are the ovisacs containing the ova, the follicles. Immediately beneath the superficial covering is a layer of stroma, in which are a large number of minute vesicles, of uniform size, about .25 mm. in diameter. These are the follicles in their earliest condition, and the layer where they are found has been termed the cortical layer. They are especially numerous in the ovary of a young child. After puberty, and during the whole of the child-bearing period, large or mature or almost mature follicles are also found in the cortical layer in small numbers, and also corpora lutea, the remains of the follicles which have burst and

are undergoing atrophy and absorption. Beneath this superficial stratum, other large and more or less mature follicles are found embedded in the ovarian stroma. These increase in size as they recede from the surface toward a highly vascular stroma in the center of the organ, termed the medullary substance. This stroma forms the tissue of the hilum by which the ovary is attached, and through which the bloodvessels enter but it does not contain any follicles.

The larger follicles consist of an external fibrovascular coat, connected with the surrounding stroma of the ovary by a network of bloodvessels, and an internal coat, which consists of several layers of nucleated cells, called the membrana granulosa. At one part of the mature follicle the cells of the membrana granulosa are collected into a mass which projects into the cavity of the follicle. This is called the discus proligerus, and in it the ovum is imbedded. The follicle contains a transparent albuminous fluid.

The development and maturation of the follicles and ova continue uninterruptedly from puberty to the end of the fruitful period of woman's life, while their formation commences before birth. Before puberty the ovaries are small and the follicles contained in them are disposed in a comparatively thick layer in a cortical substance. Here they present the appearance of a large number of minute closed vesicles, constituting the early condition of the follicles, while many never attain full development but shrink and disappear. At puberty the ovaries enlarge and become more vascular, the follicles are developed in greater abundance, and their ova are capable of fecundation.

The follicles, after attaining a certain stage of development, gradually approach the surface of the ovary and burst. The ovum and fluid contents of the follicle are liberated on the exterior of the ovary, and carried into the uterine tube by currents set up by the

movements of the cilia covering the mucous membrane of the fimbriae. After the discharge of the ovum the lining of the follicle is thrown into folds, and vascular processes grow inward from the surrounding tissue. In this way the space is filled up and the corpus luteum formed, which consists at first of a radial arrangement of yellow cells with bloodvessels and lymphatic spaces, and later it merges with the surrounding stroma.

The arteries of the ovaries and uterine tubes are the ovarian from the aorta. Each anastomoses freely in the mesosalpinx, with the uterine artery giving some branches to the uterine tube, and others which traverse the mesovarium and enter the hilum of the ovary. The veins emerge from the hilum in the form of a plexus from which the ovarian vein is formed, and leaves the pelvis in company with the artery. The nerves are derived from the hypogastric or pelvic plexus and from the ovarian plexus, the uterine tube receiving a branch from one of the uterine nerves.

PART V. Physiology.

The essential organ of reproduction in the female is the ovary, the seat of the production of the ovum. The accessory organs include the oviducts or Fallopian tubes, the uterus in which the fertilized ovum is retained during the first nine months of its development, and the vagina which is especially adapted for the reception of the male organ in the act of impregnation. Among the accessory organs we may also include the mammary glands, which undergo a special development during pregnancy, and serve for the nourishment of the young individual during the first period of extra-uterine life.

(a). The Graafian Follicle and the Corpus Luteum.

The functional value of the ovary is connected with the formation and rupture of the Graafian follicles, whereby an ovum is liberated. At birth the ovary consists of a stroma of spindle-shaped cells, and is covered by a layer of cubical epithelium continuous with the endothelium lining the general peritoneal cavity. Embedded in the stroma but especially numerous just underneath the epithelium are a vast number of primordial follicles, which are formed during fetal life by downgrowths of the germinal epithelium. The primordial follicles consist of an ovum surrounded by a layer of follicular epithelium.

Beginning at a certain time after birth and continuing throughout the period of active sexual life, some of these primordial follicles develop into mature Graafian follicles and migrate to the surface of the ovary. The change consists in a proliferation of the follicular epithelium and the formation of a serous liquid, the liquor folliculi, between the layers of this epithelium. In the matured follicle there is a connective tissue covering, the theca

folliculi, formed from the stroma of the ovary and consisting of two coats or tunics, the external and the internal. The cells in the internal tunic develop a yellowish pigment as the follicle grows, and are sometimes designated as lutein cells. Within the capsule formed by the internal tunic there is a layer of follicular cells known as the membrana granulosa and attached to one side is a mass of the same cells, the discus proligerus, within which the ovum is embedded. The follicular liquid which lies between increases in amount, and when the follicule has reached the surface it forms a vesicle projecting to the exterior. This projecting portion is nearly bloodless and thinner than the rest of the wall of the follicle and is termed the stigma. When fully mature the follicle ruptures at the stigma and the egg, together with the surrounding follicular cells of the discus proligerus and a portion of the membrana granulosa, is extruded, the egg being received into the open end of the Fallopian tube. According to Clark,* the rupture of the follicle is brought about by an increasing vascular congestion of the ovary. The tension within the ovary is thereby increased, the follicle forced to the surface, and the circulation at the most projecting portion is interfered with to such an extent as to cause necrotic changes at the stigma, at which the rupture finally occurs.

After the bursting of the follicle its walls collapse, and the central cavity receives some blood from the ruptured vessels of the theca. Later on the vesicle becomes filled with cells containing a yellow pigment. These cells increase rapidly and form a festooned border of increasing thickness around the central blood clot. The vesicle at this stage, on account of the yellow color of the new cells, is known as the corpus luteum. The structure thus formed increases in size for a period and then undergoes retrogressive changes and is finally completely absorbed. The duration of the

* 1899

period of growth varies according as the egg liberated becomes fertilized or not. If fertilization does not occur, as is the case in the usual monthly periods, the corpus luteum reaches its maximum size within two to three weeks and then begins to be absorbed. It is frequently known under these circumstances as the false corpus luteum or the corpus luteum of menstruation. In case the egg is fertilized and the woman becomes pregnant, the life history of the corpus luteum is much prolonged. Instead of undergoing absorption after the third week it continues to increase in size by multiplication of the lutein cells during the first few months of pregnancy, and does not show retrogressive changes until the sixth month or later. Recent observers agree that there is no essential difference in structure between the true and the false corpus luteum, although the former has a longer history and attains a greater size. The point of greatest structural interest in the corpus luteum is the origin of the lutein cells. Histologists have been divided upon this point, but the chief results obtained by the investigations of recent observers such as Sobotta, Stratz, Honoré, Van der Stricht, and F.H.A. Marshall, all of whom agree, may be summarized in the following manner: "The lutein cells of the fully developed corpus luteum represent the epithelial cells of the undischarged Graafian follicle. These cells, after rupture, undergo an enormous hypertrophy, which may be accompanied in the earlier stages by mitotic division, but usually only to a relatively slight extent. Meanwhile, the thickness of the wall of the developing corpus luteum is further increased by an ingrowth of connective tissue from the side of the follicle, forming eventually an anastomosis of cells, generally fusiform in shape, between the hypertrophying follicular epithelial cells. This connective tissue is derived either from the theca interna alone, or it may arise from both the theca interna and externa. The formation of the anastomosis is

accompanied by an ingrowth of bloodvessels which gradually increase in number throughout the young corpus luteum. Certain cells in this layer are considered in some cases to become transformed into lutein cells. The cavity of the discharged follicle becomes completely filled in eventually by the further growth inward of connective tissue accompanied by bloodvessels."*

Regarding the physiological importance of the corpus luteum, opinions have differed widely, but the present tendency is to attribute to the lutein cells secretory functions of the most important character. The corpus luteum is regarded as a glandular organ producing an internal secretion. The nature and precise function or functions of the secretion are under investigation. The results obtained so far indicate the possibility, on the one hand, of hormones which during pregnancy stimulate the growth of the uterus and fetus and mammary gland, and, on the other hand, inhibit the normal process of ovulation in the ovary.

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* Marshall, F.H.A. The Physiology of Reproduction. Longmans, Green and Co. 2nd Ed. New York. 1922.

PART VI. The Internal Secretion of the Ovaries.

The evidence for an internal secretion or, possibly, more than one kind of internal secretion by the ovaries is circumstantial, but convincing. The whole of the oestrus cycle, involving, as it does, periodic changes in the ovary itself, the uterus, vagina, mammary glands, and possibly in general metabolism, is dependent upon a stimulus of some kind supplied by the ovaries. If these organs are removed the oestrus cycle drops out, but if previously an ovary had been transplanted to some other region then ovariectomy does not eliminate the cycle. It may be inferred, therefore, that the stimulus furnished by the ovary is transmitted through the blood and is, therefore, probably of the nature of a hormone. The tissue elements in the ovary responsible for the internal secretion were supposed to be either the follicular epithelium or the interstitial tissue, but this latter tissue is not so constant or so evident in the ovary as in the testis, and not so good a case can be made out for its specific activity.

Much interesting evidence has been obtained of the relationship between the corpora lutea formed after ovulation and the implantation of the fertilized ovum. The implantation and normal development of the fertilized ovum fails if the corpora lutea are destroyed, and this result has been explained on the hypothesis that the luteal cells furnish a secretion which is essential to the process of implantation of the ovum.

The attention of the earlier investigators was focused for a long time almost exclusively on the corpus luteum. This was largely due to the fact that Prenant, Beard, Born, and Fraenkel regarded the corpus luteum alone as a gland of internal secretion and therefore

sought explanation of all the sex phenomena mainly in the yellow body. Furthermore, it was much easier and more feasible to shell out the corpus luteum than any other component of the ovary.

Prenant, in the Rév. Gén. des Sciences of 1898, seems to have been the first to suggest that the corpus luteum was a ductless gland. He supposed it to produce an internal secretion which exercised an influence over the general metabolism in the manner attributed to the internal ovarian secretion. The phenomenon of chlorosis was explained as being due to the absence of this secretion. Prenant also supposed that the corpus luteum had the further function of preventing ovulation during pregnancy or between the oestrus periods. This theory was supported by Regaud and Policard who stated that, by means of special methods of staining, droplets of a secretory substance could be detected in the cells of the corpus luteum of the hedge hog. Beard independently suggested that the corpus luteum was a contrivance to suppress ovulation during pregnancy, while he supposed it to degenerate before parturition. Born was the first to suggest that the function of the corpus luteum might be to provide an internal secretion which assisted in the attachment of the embryo to the uterine mucosa.

(a). The Secretion of the Corpus Luteum.

(1). Progestational Proliferation.

Fraenkel was the first to attempt to solve Born's hypothesis by experimental methods. He operated upon pregnant rabbits, removing the corpora lutea, finding with uniformity that if ablated early in pregnancy, the fetuses were absorbed, if later in pregnancy, aborted. Injury, excision or burning of other portions of the ovary did not produce this effect. Consequently Fraenkel felt justified in concluding that the corpus luteum was necessary for the nidation of the

ova and the continuation of pregnancy. Apart from the experimental evidence, Fraenkel adduced certain other facts which tend to support the theory that the corpus luteum is an organ of internal secretion. He pointed out that its general structure was suggestive of its being a ductless gland, since it is formed mainly of large epitheloid cells surrounded by a network of capillaries and arranged in regular rows or columns not unlike those of the cortex of the supra-renal body. Moreover, the increase in the size of the corpus luteum until it becomes larger than a Graafian follicle seemed inexplicable on any other view.

According to Fraenkel, therefore, the corpus luteum is a ductless gland which is renewed every four weeks during reproductive life in the human female. Its function is to control the nutrition of the uterus from puberty until the menopause to prevent it from lapsing into the infantile condition or undergoing atrophy, and to prepare its mucous membrane for the maintenance of the ovum. If the ovum be fertilized, the corpus luteum is responsible for maintaining the raised nutrition of the uterus during the first part of gestation. If the ovum be unfertilized it merely produces the hyperaemia of menstruation, and then undergoes degeneration until it is renewed in fresh positions.

The main support of the Born-Fraenkel theory, showing that the nidation of the ovum is dependent upon the presence of the corpus luteum, was supplied by another type of experiment performed by Leo Loeb. Loeb determined the exact time of oestrus in guinea pigs and rabbits by permitting sterile coitus after the Fallopian tubes had been tied. If, in a normal guinea pig or rabbit, from 2 to 9 days after coitus had taken place incisions were made into the endometrium, transitory tumor-like nodules developed which in microscopical characteristics were indistinguishable from the maternal portion of the placenta of the given species used in the experiment. These tumors

persisted for ten days and then underwent necrosis. That this deciduoma formation was due to ovarian action could be readily proved by the fact that after castration was performed no deciduomata developed. Going one step further, Loeb burned out merely the corpora lutea and then was unable to obtain deciduoma formation, though this formation was unaffected by similar or more extensive injury to any other portion of the ovary. Still more substantiation of hormone influence was adduced by the fact that auto-transplants of the uterus showed slight deciduoma formation in the transplanted portions, if the animal had functioning corpora lutea. A similar reaction could be obtained by other mechanical stimuli resembling that of the imbedding of the ovum. Foreign bodies introduced into the uterus of rabbits, for example, produced a similar stimulating effect on the endometrium and a similar deciduoma reaction.

Frank in 1911 proved that Loeb's reaction was not confined to the rabbit and guinea pig by producing deciduomata in post partum rats between 2 to 9 days after the birth of the young. Corner in 1919 fully confirmed Frank's results, likewise using rats post partum. Long and Evans in 1922 succeeded in doing the same in rats in whom inhibition of oestrus was brought about by means of mechanical stimulation of the cervix through the vagina. Teel in 1926 was able to produce deciduomata in cyclical rats by arresting the cycle by means of anterior hypophyseal fluid. In 1928 Weichert claimed that he had obtained positive results by first producing oestrus by means of an active female sex hormone extract and then sensitizing the mucosa by means of Hisaw's corpus luteum extract. If Weichert's experiments are confirmed and substantiated, this should be considered conclusive evidence, by another method of experimentation, that the corpus luteum elaborates a hormone which sensitizes the endometrium. This sensitization, as Leo Loeb showed, produces the

maternal part of the placenta, the decidua.

In 1929 F.L. Hisaw and S.L. Leonard showed by experimentation with rabbits that the progestational proliferation of the rabbit uterine endometrium seems to be the result of a combined effort of the follicular and corpus luteum hormones. Neither the follicular nor the corpus luteum extracts used were able to produce these changes when given alone. It seems as though the rabbit uterus must be under or recovering from the influence of the follicular hormone before the corpus luteum extract can act. These results lead to the conclusion that the corpus luteum extract is not capable of prolonging the progestational condition over an extended period without the presence of the follicular hormone; that is, it seems that the action of the follicular hormone is not only necessary for the beginning of the reaction by the corpus luteum extract but the follicular effect must be present if the corpus luteum extract is to prolong the progestational picture. The function of the follicular hormone would then appear to govern the growth and enlargement of the uterus, as this is the condition characteristic of oestrum, while the corpus luteum hormone modifies the structure already formed. The follicular hormone is a growth promoting substance, as indicated by the numerous mitotic figures during its maximum influence, while the corpus luteum hormone, when given alone, does not seem to have this ability.

The idea that the follicular hormone cannot by itself produce progestational proliferation is upheld by the observations of Loeb and Kountz for the pig, and Corner and Allen for the rabbit. These workers were unable to produce a progestational endometrium in these animals with the follicular hormone alone. Novak in 1928 deduced, from theoretical considerations of reproduction in general, that extracts of the corpus luteum might be expected to have little effect in the human being unless the uterus was first prepared by the injec-

tions of the follicular hormone. Very recently W.M. Allen, in conjunction with his work on the physiology of the corpus luteum, verified the work of Hisaw and Leonard and stated that "progestational proliferation can be induced regularly in the uterus of the immature rabbit by the injection of progestin, the name applied to the corpus luteum extract producing the proliferation, if the uterus is first brought under the influence of the follicular hormone. If, however, the animals are not treated with the follicular hormone before the administration of progestin, only a very small percentage of them respond to the progestin treatment." *

The function, therefore, of the follicular hormone in this case appears to be that of putting the uterus into a proper physiological condition so it can respond to the corpus luteum hormone. Neither of these substances can produce progestational proliferation in the castrate uterus when given alone. If, however, the castrate uterus is first brought into the condition typical of oestrum through the injection of the follicular hormone and is followed immediately by corpus luteum treatment, progestational proliferation results. It also seems that a quantitative relationship between the follicular and corpus luteum hormones must exist for the prolongation of the progestational picture over an extended period.

In 1930 Allen prepared a hormone from the corpus luteum that differs from the follicular hormone and which produces progestational proliferation. He has shown, in conjunction with the work of Asdell and Marshall, and Loeb and Kountz, that the follicular hormone produces none of the uterine reactions characteristic of early pregnancy, such as progestational proliferation of the rabbit's endometrium or the special sensitization of the guinea pig's uterus

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* Allen, W.M. Physiology of the Corpus Luteum. Am. Jour. Physiol. 92, 1930.

necessary for the production of experimental deciduomata. It seems certain, therefore, that the corpus luteum contains a hormone which is entirely distinct from the follicular hormone in its effects, or in the words of Corner and Allen; "The evidence is now complete that in the rabbit the corpus luteum is an organ of internal secretion which has for one of its functions the production of a special state of the uterine mucosa and that in turn the function of the proliferated endometrium is to nourish or protect the free blastocysts and to make possible their implantation. " *

(1a). The Preparation of Progestin.

The method for the preparation of progestin to be described here was devised by W.M. Allen. The corpora lutea of the sow are dissected from the ovary on the same day that they are received from the abattoir, usually while they are still warm, ground up with an ordinary meat grinder and preserved by adding 2 volumes of 95 per cent alcohol. The material is stored in this condition until ready for use and keeps for at least 10 months with no loss of activity.

Approximately 1500 grams of tissue, which have been preserved as described, are filtered through a gauze and the residue is divided into 2 or 3 equal parts and each part extracted five times with hot alcohol in a Clarke-Bloor extractor, described by Sperry, each extraction lasting for one hour. About 250 cc. of 95 per cent alcohol are used for each extraction. The mass of tissue in the bags is broken up by kneading between extractions to facilitate the penetration of the alcoholic fumes. By this method the lipids are completely removed in a fairly short time and none of the extracted material is heated above the boiling point of alcohol for more than one hour. The extraction alcohol contains a quantity of insoluble debris but

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* Corner and Allen. Physiology of the Corpus Luteum. Vol. 88. Am. Jour. Physiol.

this is not filtered off since it is removed by subsequent treatment. The alcohol is distilled off by heat with the aid of the diminished pressure obtained with a good water pump. The extraction and preserving alcohols are distilled in separate flasks since the preserving alcohol is apt to foam by reason of its greater content of water. If a good vacuum is obtained, the temperature rarely goes above 60 C. and usually not above 45 C. When the solvent has almost been completely removed, the distillation is stopped to avoid overheating.

The residues obtained from the extraction and preserving alcohols are combined and extracted 5 times with peroxide-free ether, 500 cc. being used for the first extraction and 200 cc. for each of the remaining extractions. The ether solutions from the above extractions are combined and the volume reduced to about 100 cc. by vacuum distillation. To this solution 4 volumes of acetone are added together with 10 cc. of a saturated alcoholic solution of magnesium chloride. A heavy flocculent precipitate consisting chiefly of phospholipids is formed and is removed by decanting or centrifuging. The precipitate is redissolved in ether and reprecipitated by acetone 5 times. After the first or second precipitation the precipitate does not completely dissolve in ether, a fine white precipitate remaining. It is not necessary, however, to remove this precipitate, which consists chiefly of sphingomyelin, since it is eventually removed along with the phospholipids by discarding the precipitate obtained by the addition of acetone. The acetone-ether solutions are combined and the solvents removed by vacuum distillation on the steam bath. A heavy oil is obtained which is removed from the distilling flask to a centrifuge tube with the aid of about 50 cc. of ether. The solution is centrifuged, thus removing most of the water which is carried through the above solvents together with any ether-insoluble material. The ether

layer is precipitated off and the ether is then removed by a current of warm air. The resulting oily mixture is semi-fluid and may be administered without the addition of a vehicle, providing it is made fluid by immersing the container in warm water for a short time. The yield is about 30 grams, representing 20 grams per kilogram of fresh tissue. The stability of this crude preparation is limited since several preparations have shown a loss of most of their activity in a month's time. However, if the crude substance is stored in alcohol, there is no appreciable loss even after a month's standing. The crude product may be further purified by the removal of a large amount of the neutral fat and cholesterol by freezing from methyl alcohol as described by W.M. Allen. *

(2). Inhibition of Oestrus.

Prenant, Beard, and Born considered the effect of the corpus luteum hormone inhibitory upon ovulation. Loeb agreed with this conception and offered the following experimental proof. The cyclical rate of a large number of guinea pigs was determined by means of the time at which they accepted coitus. The acceptance of coitus in these guinea pigs in whom the tubes were tied, determined the time of oestrus and ovulation. He thereupon excised or burned out all the corpora lutea in guinea pigs whose length of cycle was normal, and found that the interval elapsing before the next oestrus was definitely shortened. No such effects were produced by laparotomy or by laparotomy combined with excision or cauterization of other portions of the ovary.

In human beings ablation of the corpus luteum within 4-5 days of the expected menstruation is regularly followed by an immediate menstrual flow. This is due to the fact that the prepared endometrium

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* Allen, W.M. American Journal of Physiology. Vol. 92, page 180.

disintegrates when the female sex hormone stimulus is abruptly withdrawn by the removal of the corpus luteum. If the corpus luteum is removed earlier, menstruation is delayed because the mucosa is not yet fully charged and a new follicle must have time to ripen and burst.

Loeb's contentions have been strengthened in a more direct way by the experiments of Papanicolaou, Parkes and Bellerby, and Gley. These investigators determined the duration of the cycle in a large number of individual animals of a given species by means of the vaginal spread tests originated by Allen and Doisy, ~~and~~ which will be described later in this paper, and then attempted to influence the cycle by corpus luteum injections. Papanicolaou used guinea pigs, Parkes and Bellerby mice, and Gley rats. Their results all point to an inhibitory action on ovulation, no oestrus taking place until the injections were stopped. As the same animals may show great variations in the length of their cycles when untreated and as the cycle is readily disturbed by non-specific interventions such as handling, change of food and environment, operation, etc., these results should be accepted with some reserve.

Persistence of the corpus luteum and the consequent inhibition of ovulation has been frequently noted in the non-pregnant cow as shown by Tandler. Veterinarians under these circumstances squeeze out the corpora lutea by rectal manipulation, oestrus then promptly supervening. More recently, the effect of the corpus luteum and ovarian extracts on the oestrus of the guinea pig was studied by D. I. Macht, A.E. Stichels, and D.L. Seckinger. By means of Allen and Doisy's vaginal smear test, they observed the effect on the oestrus cycle of guinea pigs, using specially prepared water-soluble extracts of corpus luteum. They found that injections of such extracts produced inhibition of oestrus and such inhibition was

accompanied by characteristic histological findings. Frank has shown that the fetus itself does not inhibit ovulation by implanting minced rat fetuses into the peritoneal cavity of rats. The experimental teratomata grew for varying periods of time in their host without inhibiting or affecting the cycle. If the fetus is absent, as in chorion-epithelioma, ovulation is, nevertheless, suppressed. Leo Loeb removed the corpora lutea of pregnant animals, which then ovulated although the pregnancy continued.

It should be noted here that the results stated above were obtained only when an aqueous extract of the corpus luteum was used. This has been demonstrated by Corner and Allen who were able to produce the special progestational uterine endometrial reaction in castrates only when an aqueous extract of the corpus luteum was used. Therefore it seems justifiable to conclude that the aqueous fraction of the corpus luteum extract is the substance responsible for the sensitization of the uterine mucosa and the inhibition of ovulation.

From the preceding discussions it is apparent that the corpus luteum secretes two substances. The first serves the purpose of assuring sensitization of the endometrium with consequent decidual reaction, and, in the second place, an inhibitory action upon ovulation has been demonstrated. Whether the latter reaction is a true specific and truly hormonal reaction remains to be proved. It is known through experiments of Zondek and Ascheim that ovulation can be forced upon the pregnant organism by means of anterior lobe pituitary extract without the induction of abortion, and therefore this inhibitory mechanism, which may be well worth while from the point of economy, that is the saving of unnecessary follicle wastage, appears to be otherwise unessential.

(b). The Follicular Hormone.

According to Allen and Doisy, Kountz, and many others, the follicular hormone is the cause of oestrus growth and secretion and also the ultimate cause of sex instincts in the female. It is also responsible for the attainment of puberty involving probably the development of the secondary sexual characteristics. "From a consideration of the growth of the follicle, the rapid disappearance of the effects of the hormone soon after ovulation in the normal animal, and the gel state of the material which distends the newly forming corpus luteum as contrasted with the fluid form of the liquor folliculi, we consider that the follicular hormone is secreted under the influence of the ovum by the follicle cells, and that in the ultimate analysis, its formation is due to the metabolism of the ovum. Therefore we consider it the principal female sex hormone." *

A tremenduous amount of labor was expended by the early workers who were handicapped by the difficulties of testing for and assaying the active substance with the tests then available. However, recent investigators have had the great advantage of being able to use the vaginal spread test of Allen and Doisy which has minimized the time consumed, the labor expended, and the cost involved.

The way for all this work was paved by earlier researches which clarified the sex cycle in some of the lower animals. This work dates from 1889 on, when Morau, Lataste, Retterer, and others determined that the regular cyclical changes took place in the sex organs of different species. These researches came to a head with the work of Stockard and Papanicolaou, and Long and Evans in 1917 and 1922 respectively which enabled investigators to determine the exact stage of the cycle in the living animal. Finally Allen and Doisy applied this to elaborate the vaginal smear test. The researches have aimed

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* E. Allen, J. Pratt, Q. Newell, and L. Bland. Am. Jour. Physiol. 92, 1930.

to study the action of potent extracts and to determine the site of origin of the potent substance. The results may be classified under the main headings of the induction of oestrus, pseudo-pregnancy, premature maturity, the production of the mating instinct in castrates, the analysis of growth curves, the prolongation of the cycle, the anti-masculine effect, and the effect on the basal metabolism.

(1). The Vaginal Spread Test.*

Morau described the vaginal cycle in the mouse and Retterer described the changes in the rabbit and guinea pig, as did Königstein in 1917. Stockard and Papanicolaou in 1917 observed and recorded the change in vaginal contents of the guinea pig while Long and Evans in 1922 described it in the rat. It remained for Allen and Doisy in 1923 to apply this very striking cyclical change of the rodent vagina as a test for the potency, in other words, for the assay and titerage of the female sex hormone.

In either immature or castrated rats or mice, the injection of a potent extract is followed in 48 hours by the appearance of full oestrus noticeable in the uterus and the vagina. The uterine wall becomes thin, in consequence of a marked distention of the lumen by fluid. The vaginal wall thickens, becomes cornified, and then begins to desquamate.

In consequence of this cyclical reaction, marked changes in the vaginal spread of the rat and mouse can be noted. The vaginal content of the castrate, untreated mouse or rat consists of a large number of leucocytes, an occasional eliptical, epithelial cell, and a varying amount of mucus. If such a castrate, in the resting state, is injected, and according to Allen and Doisy the substance to be tested should be injected at four hour intervals for three doses, a

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* Allen, E. and Doisy, E.A. An Ovarian Hormone. Jour. Am. Med. Assn. 1923. 81, 819.

gradual change in the cellular composition of the vaginal spread appears. After 24 to 36 hours an increased number of epithelial cells, particularly of the small variety, is noted. After the lapse of 48 hours, a full reaction documents itself by the complete disappearance of leucocytes. Instead of them, the entire vaginal spread is composed of masses of non-nucleated squamous scales. A less complete reaction shows itself in the form of complete absence of leucocytes but a large number of nucleated small epithelial cells amid the squamous scales. *

Allen and Doisy have called the amount of potent substance necessary to produce a full change from the negative to the oestrual vaginal spread in a castrate rat of approximately 140 grams in weight, a rat unit. Bugbee and Simond in 1926 tested the effect of using larger or smaller rats for titration. They concluded that the following formula would obviate inaccuracies due to the effect of direct proportion of dosage to weight. This formula is rat units per cc. equals W/140 Q, in which W equals the weight of rat in grams and Q the minimal cc. that will produce oestrus.

Many discrepancies arise from the fact that different investigators select different criteria as evidence of a positive reaction. Loewe was satisfied with a percentage count; Frank and Goldberger have a scale from 0-4, anything above 2 plus counting as a positive reaction; Laqueur demands a reduction of leucocytes to a very few with at least equal presence of nucleated and non-nucleated epithelium; and Allen and Doisy full cornification.

One error arises from giving too large a dose. A full positive will then be obtained quickly, in 18 hours instead of 48 hours, and the positive reaction will continue for several days. Most workers agree

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* Allen, E. and Doisy, E.A. The Action of an Ovarian Hormone in Test Animals. Jour. Am. Med. Assn. 1923, 81, 819.

with Allen and Doisy that 3 injections, aliquot parts, be distributed over one day. Some have given the entire material in one dose, as for example, Coward and Burn, others in 3 doses at 12 hour intervals, as done by Biedl. Such variations, however, affect the outcome. European investigators, who prefer to use mice for their work, call the amount necessary to produce the oestrual changes in the castrate mouse, a mouse unit.

According to those laboratories in which accurate titration is attempted, especially according to Laqueur, it is necessary to utilize at least 5 to 10 rats or mice with a positive percentage of 75 per cent in order to titre accurately. This is to offset the difference in susceptibility of individual animals.

The technic by which the vaginal smear is obtained is of considerable importance. Many employ a small cotton swab, others a platinum loop. Frank utilized a small bent glass tube drawn to a fine tip which is fire-polished. By means of a rubber bulb a drop of water is aspirated and, after introduction of the tip into the vaginal canal, the water is repeatedly injected and reaspirated in order to obtain a uniform sample of the vaginal contents. A cannula is used only on one animal. The used cannulae are cleaned by soaking in lye and washed before re-using. Even under these conditions, unless due care is exercised, the sample may be misleading, being positive in one area and negative in another.

Bugbee and Simond attempted standardization by means of the vaginal spread of rats. Their formula for the rat is described on the preceding page. This is a convenient but not strictly an accurate method. If real accuracy in titration is attempted, Laqueur's method of employing numerous animals is preferable.

Errors resulting from reformation of ovarian tissue adhering to the bursa ovarii of rats and more particularly of mice, must be care-

fully guarded against and kept in mind when positive results occur. Davenport reported that there was the reformation of ovarian tissue after apparently careful castration performed on mice in approximately 22 per cent. Laqueur and others have noted this same reformation with subsequent spontaneous recurrence of oestrus. Loewe has suggested that mice, after castration, should be watched for 44 days before using them for injection and assay, in order to avoid errors from this source, as spontaneous oestrus always documents itself before the elapse of six weeks. Frank has demonstrated that, by removing a large area of the peritoneum and Fallopian tubes together with the ovaries, reformation of ovarian tissue has become a very rare occurrence. However, if a positive reaction is obtained, vaginal spreads of such mice or rats are followed for a period of 10 days in all experimental work, in order to exclude even the small possibility of spontaneous oestrus.

(2). Induction of Oestrus.

At the same time that Allen and Doisy, in 1923, published their vaginal smear test, they described the effects produced by injecting follicle fluid and lipoid extracts of follicle fluid. Every stage, from anoestrus to oestrus, could be reproduced both in the vaginal spread and in the vaginal walls and uterus of castrated rats. The same investigators were able to produce oestrus in castrated mice as well as to produce menstruation in castrated monkeys.

Courrier in 1924, by means of follicle extract and follicle fluid, produced full oestrus in a guinea pig. In 1926 he reported similar results with castrated rabbits. Courrier likewise showed that the regressive changes in the Fallopian tube of the castrated rabbit were promptly relieved by injections of the female sex hormone. Hartman in 1926 produced oestrus in the castrated opossum by means of follicular fluid extracts. These authors also obtained equally striking results with placental extracts.

Frank and Gustavson in 1925 reported full oestrus effects by means of corpus luteum extracts derived from hogs and cattle. They examined various batches of corpora lutea of different ages and found that at the beginning of the involutionary stage the corpora contained the most female sex hormone. From their investigations they determined that the follicle secreted female sex hormone to the time of ovulation. From then on, in the fertile cycle, the corpus luteum continued the secretion at least until the placenta took on this essential task. In order to properly designate this continuous relay action of the follicle, corpus luteum, and placenta, they called this triad the "Gestational Gland". Loewe, as well as Brouha and Simonnet, have accepted this interpretation.

Pratt and Allen have obtained female sex hormone in the corpus luteum of the human female. Zondek and Ascheim, by means of their implantation method, found that the corpus luteum of the human female, before menstruation, was active; after menstruation, inactive. In other words, menstruation occurs when the corpus luteum no longer secretes the hormone. They found that the pregnancy corpus luteum was active for several months of gestation, as far as its female sex hormone content is concerned. Recently Frank, Gustavson, and Goldberger have obtained both female sex hormone and sensitizing hormone from the same batch of corpus luteum.

The most recent work in this field was performed last year by E. Allen, Pratt, Q. Newell, and L. Bland. They made analyses of the hormone content of isolated tissues of the human ovary by injections of fresh follicular fluid and by implantation of fresh corpora lutea, of follicle walls and of cortical stroma free from macroscopic follicles. The test used as a criterion of hormone activity was the full oestrus reaction of the ovariectomized rat as reflected in the cell content of the vagina. Therefore the hormone sought was that responsible for the

genital growth reactions of the periods of oestrus in lower animals and of the intermenstruum of primates. They obtained high yields of this hormone from recent corpora lutea removed from the 13th to the 17th day of the menstrual cycle. There was a considerable decrease in amount by the 22nd day. Corpora of the first third of gestation contained considerable amounts while two full term corpora gave negative results. This last result indicates that toward the end of gestation the corpus luteum of woman does not share responsibility for the large amounts of this hormone in the placenta, blood, and urine. They also found that the liquor folliculi and pieces of the follicle wall, mostly granulosa cells, contained considerable amounts of this hormone. Cortical stroma without macroscopic follicles, taken from ovaries containing follicles or corpora in which the hormone was demonstrated, gave negative results.

These results added to earlier data demonstrate that, as far as the secretion of this hormone is concerned, the human corpus luteum merely continues an activity which is primarily follicular. The hormone content of the human corpus luteum is highest just after ovulation in the early stages of transition from the follicle to the corpus luteum and decreases before the onset of the next menses. Normal stroma tissue of the ovarian cortex shares little in this function.

Increasing evidence has accumulated, at a rapid rate in the last few months, that the contention of the earlier investigators that a special secretion or special secretions are elaborated by the corpus luteum, holds true. The corpus luteum forms the link, in what has been called the "Gestational Gland", namely a compound and progressive gland of internal secretion, consisting of the growing follicle, the corpus luteum, and the placenta, all three of which, during their period of activity, elaborate and pour into the circulation female sex hormone.

Quite distinct from this secretion and analagous to the compound and multiple nature of other glands of internal secretion, such as the anterior lobe of the pituitary, the corpus luteum elaborates another secretory product. This, mainly water soluble incretion, has the function of sensitizing the mucous membrane of the uterus during the pregravid stage in order that it may respond to the irritation and invasion of the fertilized ovum by the formation of the maternal placental elements. Without this sensitization, the ovum cannot successfully take root and flourish. In addition to this influence, the aqueous corpus luteum secretion likewise inhibits the growth of follicles and thus, under normal conditions, helps to produce the periodicity of the cycle.

In accord with the evidence presented, therefore, we may accept the presence of two sex hormones, the one, mainly fat soluble, the female sex hormone produced by the ovary, the corpus luteum, and the placenta; the other, mainly water soluble, elaborated by the corpus luteum alone.

(3). Other Effects Produced by the Female Sex Hormone.

(1a). Induction of Premature Puberty.

Many investigators, including the pioneers, have shown that immature animals can be made sexually mature by injections of active hormonal extracts obtained from the ovary or placenta. Allen and Doisy, in 1923, described the induction of premature maturity in rats and mice. In these animals the onset of puberty is strikingly shown by the opening of the vagina, usually followed in a few days by an oestrus smear. They found that if the injections with the active substance were stopped, these animals had no further cycle until the normal time of puberty arrived. Frank, Kingery, and Gustavson, in 1925, induced premature puberty in a number of rats, and shortly after the cessation of injection, noticed that some of these immature animals

continued to have cyclical manifestations until the cycle merged with that of the normal time of puberty. It is possible that the spontaneous continuation of oestrus may have resulted through the presence of unsuspected anterior lobe pituitary extract rather than from the female sex hormone. However, investigation of the ovaries of these immature animals did not demonstrate the hyper-ovulation and luteinization which is regarded as a pituitary effect. Laqueur, in 1927, published an extensive study on the increase in size and weight of the genitals in rats in which premature puberty had been induced. The increase in weight in certain instances reached 300 per cent. He also obtained these effects in guinea pigs, mice, and bitches. This study fully confirmed the work of early authors.

(2a). Induction of the Mating Instinct.

Frank, in 1911, showed that pregnant rabbits will accept a vigorous male. This was verified by Hammond and Marshall in 1923. Allen and Doisy, in their earliest publication, emphasized the fact that the follicle extract reactivated the mating instinct of castrated rats. In a later publication, they stated that copulation was noted in 7 of 11 castrates receiving injections. Other workers recently have found that the active substance, whether obtained from the follicle, the placenta, or the corpus luteum, has an identical effect in this regard.

(3a). Prolongation of the Cycle.

Frank and Rosenbloom discovered that continued injection of sufficient amounts of active substance would maintain the anabolic changes of Muller's tract and of the breasts indefinitely if sufficient material was used. Hugh hyperplasia corresponding to immediate post partum conditions resulted throughout the experiment, some of which were continued for 30 days in rabbits, guinea pigs, and rats.

Allen and Doisy maintained rats for at least two weeks in full oestrus without the appearance of any leucocytes in the spreads.

Laqueur was able to keep normal mice in full oestrus continuously for six weeks by a daily dose of two mouse units.

(4a). Breast Increase.

All of the earlier investigators, including Aschner, Schickele, Herrmann, and Frank and Rosenbloom, obtained increase of the breasts and nipples of immature as well as castrated animals by means of a potent extract. It is well to remember that these growth changes occur physiologically in connection with the first oestral phenomena. The growth of the main duct system is permanent, that of the terminal ducts and alveoli, showing a cyclical variation.

Both Tsu-Zong-Yung, in 1924, and especially Vintemberger, in 1925, studied the stimulating effect of the follicle fluid, in particular, on the breasts of rabbits. Frank has found the results, whether obtained by means of follicle fluid, corpus luteum extract, or placental extract, identical. Laqueur in 1927 again drew attention to the breast phenomena, employing female and male rats. Lipschütz as well as Steinbach utilized the breast growth in males as an indication of successful "hyperfemininization" which they produced by means of ovarian transplants or injection with the female sex hormone. It would seem that the breast is an indifferent tissue, a bisexual gland, which, however, responds by anabolic changes to the female sex hormone.

Hartman has recommended the inspection and palpation of the breasts of castrated opossums as a biological index of the effect of the female sex hormone. In this marsupial, the nipples and mammary glands are concentrated in a portion of the abdominal pouch and therefore readily lend themselves to palpatory examination.

(5a). Effect on the Basal Metabolism.

Loewy and Richter, in 1899, found that the basal metabolic rate of women was reduced by castration. They reported an increase following the use of desiccated ovarian substance. Zondek and Bernhardt,

in 1925 employed freshly dried pig's ovaries on a woman castrated two years previously and found that the basal metabolism was raised 12.4 per cent.

Laqueur has performed a series of experiments which are convincing in their nature. He made accurate gasometric measurements of the oxygen and carbon dioxide consumption of 6 mice simultaneously. The investigation was pursued daily over two or three weeks, each part of the experiment lasting for three hours. It was found that an increase of basal metabolism resulted from the injection of female sex hormone into female castrates. No such effect could be obtained in the male castrate with female sex hormone.

Apparently, therefore, there should be no further doubt that castration, on one hand, is uniformly followed by a slight diminution in basal metabolism, and that on the other hand, the injection of female sex hormone again raises the basal exchange to a normal level, and that this effect is sex specific.

(6a). Effect on the Ovaries.

All the earlier investigators, with the exception of Herrmann and Stein, agreed that injection of the active substance had no effect on the ovaries of either immature or mature animals. The more recent investigations have sustained this contention with the exception of Laqueur who mentions that the ovaries contain less follicles after female sex hormone has been exhibited for any length of time.

This lack of influence on the female gonad itself is in marked contrast to the rapid and striking activation of follicle ripening and luteinization which results from injection of anterior pituitary lobe extracts or implants in both premature and adults.

(4). The Female Sex Hormone in the Blood.

In 1905 Marshall and Jolly injected the blood of oestrus bitches into other anoestrus bitches, and claimed to have obtained immediate

flushing of the vulva in the anoestrus animal. Fellner in 1913, using 5-10 cc. of pregnant rabbit's blood, injected a 2500 gram rabbit, obtaining doubtful results on the uterus. F. Binz in 1924 published a small communication dealing with the production of signs of premature puberty in infantile mice following injection of the blood serum of pregnant women. Loewe in 1925 announced the recovery of active substance obtained from the blood of a female rabbit, cow, and woman. The activity was demonstrated by the Allen and Doisy test. About the same time Frank, Gustavson, and Weyerts announced the recovery of the active substance from the blood of oestrus sows, its absence in the non-oestrus animal, and in bull's blood.

Since 1925 Frank and Goldberger have studied the variation of the female sex hormone in the circulating blood of women at different stages of the cycle, establishing norms. They also described the continuation of a high hormone level in the circulation from the 7th week of pregnancy on to term. They have also demonstrated that the menstrual blood contains a concentrated amount of female sex hormone.

(5). The Female Sex Hormone in the Urine.

Loewe in 1926 discovered the female sex hormone in the urine. He found that during the cycle, at about the 13th day after the onset of the previous menstruation, at most one to two mouse units per liter could be obtained. Laqueur, on the other hand, reports finding as much as 200 mouse units per liter on the second day of the menses. Frank believes that at the time of impending menstruation the kidney and intestinal threshold for the female sex hormone is abruptly lowered.

Zondek* discovered hugh amounts of hormone in the urine of pregnant women. This excess of excretion was found from about the eighth week on, extending to term. Throughout this time, from six to seven

* 1928

thousand mouse units, with a maximum of ten thousand mouse units per liter, were noted.

Mazer and Hoffman, in 1929, attempted to diagnose pregnancy in the early weeks by injecting 20 minims of freshly catheterized urine, every 2 hours for 10 hours, into castrated mice. A positive vaginal spread was regarded as evidence of pregnancy. In 67 tests on pregnant women 61 were positive, the negative being in the first five weeks of gestation. Of 142 control tests, 15 were positive.

It is thus apparent that an enormous overproduction of female sex hormone takes place throughout pregnancy. For this reason Zondek and Ascheim have proposed the urine of pregnant women as a convenient source for obtaining the hormone.

(6). The Nomenclature of the Hormone.

The utmost confusion has prevailed in the nomenclature applied to the female sex hormone and substances giving this reaction. This was largely due to the fact that the active substance had not been isolated and that investigators have been inclined to name the active fractions according to the tissues from which derived.

The earliest investigators called the supposedly active substance "ovarin" (Poehl), "oöphorin" (Landau), "biovar, protovar, and luteovar" (Okinschitz) which all refer to the derivation. On the other hand, Seitz, Wintz, and Fingerhut called their product "sistomensin", believing that this influenced the menses to subside, and "agomensin", a fraction which was to stimulate the menstrual flow. Schickele, Aschner, Frank, and others were content to call their active fraction "corpus luteum" containing, or "placenta" containing hormone, and later Frank spoke of "follicle fluid" as such. As early as 1911, G. Klein used the word "folliculin" which was taken up by others.

"Feminin" and "gynacin" (Glimm and Wadehn) have likewise been

applied to an active fraction. In 1923 Allen and Doisy spoke of an "ovarian" hormone, "follicular" or "oestrus" hormone, all applied to the same active substance. Frank, Gustavson, and many others have used the noncommittal term, "female sex hormone". Parkes and Bellerby spoke of "oestrin" whether derived from the follicle or placental material.

Laqueur called an active fraction which is water soluble and contains not more than .01 mgm. of solids per mouse unit, "menformon". Loewe, in 1926, suggested the inclusive nomenclature of "thelykinin", "thelys" signifying "the feminine", and "kineo" I set going. As subtitles he gave "thelystasin", the inhibiting portion, and "thelytropin", a combination. Blotevogel, Dohrn, and Poll suggested the name of "tokokinin" which signifies the procreative hormone applicable to both the male and female.

In 1930, however, this hormone was isolated by E. Doisy and was approved by the American Medical Association. *"In view of the many names suggested by investigators who have worked with the ovarian hormone which produces oestrus in the ovariectomized female, it was difficult to select a name for the pure crystalline product. Such names as folliculin, oestrin, etc. appeared to be satisfactory in many respects but could not be accepted because they had already been utilized as trade names by pharmaceutical houses for extracts containing only the partially purified hormone. The isolation of the new crystalline hormone seemed to justify the selection of a new name which should be reserved for the crystalline material and which in reality would become the name of a new and probably hitherto unknown chemical substance." The term "theelin" was suggested which is derived from the Greek word "theelus" used both adjectively and nominally

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* Doisy, E., Veler, C., and Thayer, S. The Preparation of the Crystalline Hormone Theelin. Jour. Am. Med. Assn., 1930.

to signify female.

The term "theelin" was adopted after a discussion of possible names by E. Doisy with Dean A.M. Schwitella of the St. Louis University School of Medicine and Dr. N.M. Leech of the American Medical Association laboratories. Owing to the possibility that solutions of the crystalline hormone may be used clinically, this name was submitted to the Council on Pharmacy and Chemistry, American Medical Association, and was approved.

(7). A Brief Survey of the Preparation of Theelin.*

Essentially the procedure depends upon the fact that theelin, which behaves as a very weak acid, may be extracted from organic solvents with dilute sodium hydroxide solutions, and then in turn may be extracted from the alkaline solution by certain organic solvents.

The chief source of the substance is the urine of pregnant women. The outline of the process is as follows:

1. Urine--butyl alcohol extraction in continuous liquid extraction.
2. Butyl extraction distilled; residue leached with benzene. Insoluble residue discarded--3.2% loss.
3. The solution is distilled and residue transferred to solution in dilute sodium hydroxide. Insoluble tar discarded--4.2% loss.
4. Alkaline solution is extracted with ethyl ether.
5. Ether extraction is distilled; residue is steam distilled.
6. Residue is leached with hot 0.25 N sodium hydroxide. Residue discarded--1% loss.
7. Solution extracted with ethyl ether; then ether distilled.
8. Residue leached with cold 0.25 N sodium hydroxide; insoluble residue discarded--9% loss.
9. Solution extracted with ether and ether distilled.
10. Residue crystallized from hot 25% ethyl or butyl alcohol.

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* Footnote on page 40.

(8). Organotherapy.

It is a matter of interest that as early as 1893 Regis of Bordeaux made an extract of ovaries which he injected for the cure of insanity following upon operative menopause. Landau in 1896 used desiccated ovaries for the treatment of castration and menopause symptoms. Poehl of St. Petersburg prepared both powdered and aqueous extracts of desiccated ovaries. Since then such preparations have been marketed by innumerable firms and no uniformity of preparation has been observed. In some instances, the material has been defatted in order to make it more palatable, less disturbing to the digestion, and less easily decomposed. At present most pharmaceutical houses attempt to perform all processes in vacuo and at a low temperature.

The effects on the basal metabolism of oöphorin and similar desiccated ovarian powders have never given uniform results and have not stood the test of careful scrutiny and repetition. Frank tested a number of marketed preparations biologically and found them wanting in efficacy. Geist and Harris found the same thing. Loewe was unable to obtain any biological reaction in corpus luteum preparations purchased or which he himself prepared. Zondek and Bernhardt too were unable to find any activity in the marketed preparations they tested.

However, literature teems with the glowing reports of the efficacy and wonderful results obtained with these biologically inert preparations. These effects vary from the immediate induction of menstruation after long periods of amenorrhea, the complete abolition of annoying and distressing menopause symptoms such as flushes, dizziness, and sweats, to the complete relief of intractable vomiting of pregnancy.

The following desiccated ovarian preparations were tested by both the growth of the rabbit's uterus and the vaginal smear method. Hugh doses were given subcutaneously and in the case of tablets, the latter

were finally powdered, emulsified with water, and injected.

Pharmaceutical Preparations Tested.*

Armour's ovarian substance-neg.
Burroughs, Wellcome, "Varium"-neg.
"Ciba" Sistomensin and Agomensin-neg.
Hynson, Westcott's Lutein tablets-neg.
Iscovesco's Gynocrinol-Hypo-neg. Mouth tablets-neg.
Lederle's Corpus Luteum sol.-neg.
Lehn & Fink, Ovarian substance-neg.
Parke, Davis & Co.-ampoules corpus luteum-neg., ovarian sub.-neg.
Reed & Carnrick Ovocoid Pills-neg.

A number of patents have been taken out based on methods of obtaining the female sex hormone. None of them contains anything of importance or value. It is of interest to note some of them here.

George M. Hieatzman, of Baltimore County, William I. Hieatzman, of Baltimore, Maryland. Process of Separating Ovarian Product from its Surrounding Membrane. 1,163,538. Patented Dec. 7, 1915. U.S.P.

Ludwig Seitz and Herrmann Wintz, of Erlangen, Germany. Process for the Manufacture of a Menses-Increasing Substance from the Corpus Luteum. 1,318,698, and 1,318,699. Patented Oct. 14, 1919. U.S.P.

Sigmund Frankel and Edmund Herrmann, of Vienna, Austria. Hormones and Phosphatides and Process of Obtaining Same. 1,314,321. Patented Aug. 26, 1919. U.S.P.

Frank, Goldberger, and Gustavson have obtained the following results in a study of the extracts for which claims of biological titration have been made.

Marketed By	Trade Name	Labeled Rat or Mouse Units Per cc.	Latter Found.
Squibb	Ovarian Hormone	3 Rat Units	1
Squibb	Amniotin	25 Rat Units	3-0
Parke, Davis	Estrogen	25 Rat Units	2-0
Degewop	Menformon, old	4 Mouse Units	0.75
Degewop	Menformon, new	40 Mouse Units	0
Ciba	Sistomensin	6 Rat Units	0

As will be seen from the above, these titrations are extremely

* Frank, R.T., Gustavson, R.G., Hyndman, D., and Kreuger, H. The present chemical status of the female sex hormone. Endocrin. 1926, 10:260.

disappointing, showing that even if the material left the hands of the manufacturer with the amount of mouse or rat units labeled, deterioration destroys most of the activity and makes the clinical use illusory.

At present the prices of the various ovarian hormones and female sex hormones are almost prohibitive. The new Folliculin of which each ampoule is said to contain 40 mouse units, has been sold at $5.00 for 3 ampoules. Ten tablets of Progynon, each tablet presumably 250 mouse units, cost $9.50. Estrogen costs $4.00 for 6 ampoules, each ampoule labeled 25 mouse units, Amniotin, labeled 50 units, $3.00 for 5 cc., and the latter labeled 100 units, $6.00 for 5 cc.

A study of the newer preparations offered to the profession in the last few years has been made by Frank and Goldberger.* These two men have been untiring in their work in the field of ovarian organotherapy, and I will finish this chapter on organotherapy by quoting from their recent studies. "No claim at minutely accurate titration is made. However, our results are sufficiently conclusive to warrant our pointing out that the preparations now obtainable do not as yet meet the needs of practice, nor do they fulfil the requirements of the most lenient possible standard that we may set up. None of the preparations, to use a comparison, approaches what we require and demand in thyroid therapy or in the treatment of diabetes. Everything points to the fact that before much time has elapsed, the concentrated and continuous effort of many investigators will produce a usable and valuable female sex hormone preparation."

* 1928-29

PART VII. The Summary.

The follicular or female sex hormone is the cause of oestrus growth and secretion and also the ultimate cause of sex instincts in the female. It is also responsible for the attainment of puberty involving probably the development of the secondary sex characters.

The way for all the work on the female sex hormone was paved by the earlier researches which clarified the sex cycle in some of the lower animals. This work dates from 1889 on, when Morau, Lataste, as well as Retterer, 1892, and others, including Heape, 1899, determined that regular cyclical changes took place in the sex organs of different species. These researches came to a head with the work of Stockard and Papanicolaou, 1917, and Long and Evans, 1922, which enabled investigators to determine the exact stage of the cycle in the living animal. Finally Allen and Doisy applied this to elaborate the vaginal smear test. The latter test has minimized the time consumed, the labor expended, and the cost involved in the study of the female sex hormone.

The follicle, corpus luteum, and placenta secrete the female sex hormone. The hormone content of the corpus luteum is highest just after ovulation in the early stages of transition from the follicle to the corpus luteum and decreases before the onset of the next menses. In pregnant women, the corpus luteum continues the secretion until near the end of the period of gestation. The corpus luteum forms the link in what has been called the "gestational" gland, namely a compound and progressive gland of internal secretion, consisting of the growing follicle, the corpus luteum, and the placenta, all three of which, during their period of activity, elaborate and pour forth into the circulation female sex hormone.

Quite distinct from this secretion and analagous to the compound and multiple nature of other glands of internal secretion, such as the

anterior lobe of the pituitary, the corpus luteum elaborates another secretory product. This mainly water soluble incretion has the function of sensitizing the mucous membrane of the uterus during the pregravid stage in order that it may respond to the irritation and invasion of the fertilized ovum by the formation of the maternal placental elements. Without this sensitization, the ovum cannot successfully take root and flourish. In addition to this influence, the aqueous extract of the corpus luteum likewise inhibits the growth of follicles and thus, under normal conditions, helps to produce the periodicity of the cycle.

We may, therefore, accept the presence of two sex hormones, the one, mainly fat soluble, the female sex hormone produced by the ovary, the corpus luteum, and the placenta; the other, mainly water soluble, elaborated by the corpus luteum alone.

The chemical researches on the female sex hormone were up to last year(1930) very disappointing. However, in 1930, Doisy isolated the female sex hormone in crystalline form. The product was termed "theelin" and was approved by the American Medical Association. However, it has not yet been placed on the market. The female sex hormone is of the simple composition(C,H,O). Chemically it shows lack of affinities and activity; biologically, on the contrary, its activity is exerted at minute concentration.

"We are justified in ascribing the phenomena of sex and reproduction to two hormones, the general female sex hormone elaborated by the "gestational" gland, and the more specific and less widely distributed, but for that reason not less important, corpus luteum hormone whose activity is limited to imbedding of the ovum and regulation of follicle growth. The effect produced by the female sex hormone fosters, matures, and stimulates all feminine qualities and attributes, as well as the

organs needed for the perpetuation of the species."*

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* Dr. R.T. Frank, Mt. Sinai Hospital, New York City. 1928-29

PART VIII.

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